

THE DISTRIBUTION OF PHOSPHORUS (P^{31} AND P^{32}) IN DORSAL AND VENTRAL HALVES OF THE RANA PIPIENS GASTRULA¹

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Studies of the distribution of enzymatic activity and oxygen consumption in the amphibian gastrula have led to hypotheses concerning the role of metabolic gradients in development (Child, 1941). Although considerable confusion exists as to whether such gradients have been established, the appearance of the dorsal lip does delineate a region of distinct morphogenetic activity from one presumably less active (the ventral half), and does suggest that a comparison of their respective metabolic activities would reflect these morphogenetic differences.

Considering the important biological and metabolic role of phosphate compounds, it was felt that a study of their distribution between these two morphogenetically distinct regions would reveal specific metabolic differences of a more convincing nature. Furthermore, radioactive phosphorus was used to make possible an analysis of shifts of phosphorus, either from one region to another, or from one component to another.

METHODS

Rana pipiens females were weighed and injected with pituitary glands to induce ovulation. They were then injected intraperitoneally with approximately 0.1 mc. of P^{32} in the form of H_3PO_4 . Forty-eight hours later, the eggs were harvested and fertilized. They were allowed to develop in large finger bowls at 15° C. until Shumway stage 10.

The jelly and vitelline membrane were removed and the gastrulae were then dissected into two halves as shown in Figure 1. Dorsal and ventral halves were collected in separate stender dishes standing in an ice water bath. From twenty to forty halves were transferred to 12-ml. graduated centrifuge tubes and washed twice with full strength Holtfreter's solution. All operations were carried out in full strength Holtfreter's solution in vessels kept in ice water and the homogenization and extraction were completed in a 4° C. cold room.

The fractionation procedure was a modified Schmidt- Thannhauser extraction (1945). The fractions isolated were the following: (1) total acid-soluble phosphorus, (2) "desoxyribonucleic acid phosphorus," (3) "ribonucleic acid phosphorus," (4) "phosphoprotein phosphorus," (5) "phospholipid phosphorus," and (6) residue phosphorus.

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After alkali digestion of the defatted material, the supernatant was poured off, DNA was precipitated by the addition of an HCl-TCA mixture and DNA phosphorus determined according to the procedure outlined by Sze (1953). The residue remaining in the alkali digest was analyzed as "residue phosphorus."

After precipitation of DNA, the remaining supernatant was precipitated with magnesia mixture overnight to obtain the inorganic phosphorus liberated from phosphoprotein. The resulting filtrate was hydrolyzed in 60% perchloric acid and analyzed as "ribonucleic acid phosphorus."

All fractions isolated were hydrolyzed in 60% perchloric acid in a sand bath until clear, and inorganic phosphorus was precipitated as the magnesium ammonium complex with magnesia mixture. The precipitates were collected on filter paper and mounted on brass discs for counting, which was done with a Geiger-Muller end window tube (3.3 mg./cm.²) using a Nucleonic RC 2 scaler. All samples were corrected for decay and the instrument was checked daily against a standard beta source.

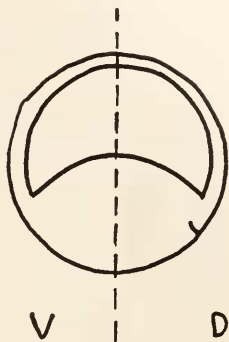


FIGURE 1. Dissection of gastrula. V = ventral half; D = dorsal half.

The precipitates were eluted in 1 N sulphuric acid and the phosphorus determined by the method of Berenblum and Chain (1938) using the vessel described by Wiame (1947).

Six separate experiments were completed, each run in duplicate, with appropriate reagent blanks.

The fractions isolated represent heterogeneous groups of phosphate compounds with a wide range of different origins (Grant, 1953). Furthermore, there is considerable doubt as to the extent of purity of these isolated fractions, particularly those fractions that may be contaminated with inorganic phosphorus (Davidson *et al.*, 1951). Because of the relative nature of the data, however, it was assumed that any significant differences between dorsal and ventral halves should be evident using this technique.

RESULTS AND DISCUSSION

In Table I, the distribution of phosphorus (P^{31} and P^{32}) in dorsal and ventral halves of the gastrula is shown, in absolute values and in percentages of total phosphorus and total radioactivity. No significant differences are evident in any of the fractions. In two experiments, whole gastrulae were extracted along with

the halves to determine the efficiency of recovery, which was fairly good. The low recoveries of acid-soluble phosphorus and phospholipid phosphorus may be attributed to loss of blastocoel fluid in the case of the former and loss of yolk granules during the dissection procedure in the case of the latter. The data do illustrate that dorsal and ventral halves have a similar distribution of phosphorus and that cleavage produces a uniform apportionment of the egg constituents.

In a recent study of the regional chemical differences in the frog gastrula (Barth and Sze, 1953), gradients of lipid and of total nitrogen (animal-vegetal) were demonstrated; however, no dorso-ventral gradient was evident, which agrees with the absence of a phosphorus gradient shown in Table I. Although the analyses of Barth and Sze were performed on several small regions of the gastrula, their data, calculated on the basis of dorsal and ventral regions (to make them approximately equivalent to half-gastrulae analyzed here), exhibit no dorso-ventral differences.

TABLE I

Distribution of phosphorus (P^{31} and P^{32}) in whole gastrulae and in dorsal and ventral halves

Fraction	Whole			Dorsal half			Ventral half		
	$\mu\text{gms. } P^{31}$	% total P^{31}	% total P^{32}	$\mu\text{gms. } P^{31}$	% total P^{31}	% total P^{32}	$\mu\text{gms. } P^{31}$	% total P^{31}	% total P^{32}
Acid-soluble P	77.0	5.35	90.00	32.5	5.10	86.74	30.5	4.34	87.58
Ribonucleic acid P	45.0	3.18	1.03	20.0	2.82	2.82	25.5	3.65	2.33
DNA P	5.0	0.39	1.34	2.5	0.40	1.23	2.0	0.33	1.14
Phospholipid P	283.5	19.65	4.78	112.8	17.47	5.79	127.7	18.12	6.09
Phosphoprotein P	974.5	67.55	2.41	489.5	73.68	4.02	517.3	72.81	3.20
Residue P	58.0	3.95	0.18	15.0	3.46	0.75	23.5	4.38	0.97

Values for $\mu\text{gms. } P^{31}$ expressed as micrograms of phosphorus per 100 embryos or per 100 half-embryos. Values for % total P^{32} obtained from values expressed as counts per minute per whole or half-embryo.

The values for all fractions, except DNA phosphorus and RNA phosphorus, compare closely with Kutsky's (1950) results. The low values for these latter two fractions may have been due to a failure to obtain complete precipitation with magnesia mixture, since the amounts involved are relatively small. In addition, loss of RNA phosphorus may have been due to adsorption onto the magnesium ammonium precipitate of phosphoprotein phosphorus. However, the relative values are significant and these indicate that no differences exist.

The per cent distribution of P^{32} also illustrates that no significant differences are apparent when the halves are compared to each other, or to the whole embryo.

When the number of cells in the two regions is considered (Sze, unpublished data), Table II is the result. Since the ventral half contains fewer, larger cells (approximately 15,800 cells with an average volume of $67,500 \mu^3$ compared to the dorsal half with 18,100 cells with an average volume of $52,600 \mu^3$) the results are to be expected. The larger ventral cells contain a greater proportion of cellular constituents, particularly yolk granules, which represent about 70% of total egg phosphorus (Grant, 1953). Thus, phosphoprotein phosphorus and phospholipid

TABLE II

Distribution of phosphorus (P^{31}) per cell of half gastrulae

Fraction	Dorsal	Ventral
Acid-soluble P	0.178	0.193
Ribonucleic acid P	0.109	0.161
Desoxyribonucleic acid P	0.014	0.013
Phospholipid P	6.155	8.075
Phosphoprotein P	26.750	32.750
Residue P	0.082	0.149

Values expressed as micrograms P^{31} per cell $\times 10^6$.

phosphorus, the major constituents of yolk phosphorus (Panijel, 1950), exhibit the greatest differences.

The specific activity data (Table III) suggest that differences between dorsal and ventral halves may exist. However, these differences are insignificant when tested by the comparison of individuals method. The high specific activity of the acid-soluble fraction in the whole embryo may be attributable to the retention of blastocoel fluid, possibly rich in highly active inorganic phosphorus.

It is possible that differences could be made more evident (that is, if they exist) if smaller regions of the gastrula were compared; regions similar to those analyzed by Barth and Sze (1953). The dissection into half gastrulae includes large areas of tissue of similar metabolic activity such that small differences between halves are masked. Possibly, in later stages of gastrulation, where metabolic differences are more pronounced (Brachet, 1950), dorsal and ventral halves would exhibit divergencies in their phosphorus distribution and specific activity.

The residue phosphorus exhibited activities of the same order of magnitude as the ribonucleic acid fraction, suggesting that it might be undigested nucleic acid. It is also possible that the residue might be metaphosphate as described by Wiame (1947) in yeast. The possible existence of metaphosphate is interesting in the light of recent findings by Berg (unpublished data) that there is a strong metaphosphatase present in the developing embryo.

No specific activity values are reported for DNA phosphorus since those experiments which yielded activities for this fraction failed to yield detectable amounts of phosphorus.

TABLE III

*Relative specific activity $\times 10^6$.
Whole and half gastrulae*

Fraction	Whole	Dorsal	Ventral
Acid-soluble P	202.25	142.48	141.72
Ribonucleic acid P	4.15	7.16	4.39
Desoxyribonucleic acid P			
Phospholipid P	2.98	2.78	2.38
Phosphoprotein P	0.38	0.43	0.31
Residue P	0.58	3.43	5.09

Values expressed as $\frac{\text{cts./min./}\mu\text{gms. P}}{\text{act. injected/wt. gms.}}$

Kutsky (1950) reports significant shifts in activity from gastrulation to neurulation. These have also been found by the author (unpublished data). This points to the need for continued study of more advanced stages of gastrulation to demonstrate and localize these changes. This report represents the completed portion of such experiments now in progress.

SUMMARY

1. A preliminary investigation of the distribution of phosphorus in dorsal and ventral halves of the *Rana pipiens* gastrula was undertaken to demonstrate the possible existence of a metabolic gradient of phosphate compounds correlated with the apparent morphological gradient. Radioactive phosphorus was employed to permit an analysis of shifts of phosphorus.

2. A modified Schmidt-Thannhauser extraction procedure was applied to dorsal and ventral halves of stage 10 (Shumway) embryos obtained from a frog injected with approximately 0.1 mc. of P^{32} before inducing ovulation. Total acid-soluble phosphorus, RNA phosphorus, DNA phosphorus, phosphoprotein phosphorus, phospholipid phosphorus and a residue phosphorus were extracted and analyzed for specific activity.

3. The distribution of phosphorus (P^{31} and P^{32}), expressed either as micrograms P^{31} per half embryo or as per cent of total P^{31} or P^{32} , exhibited no significant difference between dorsal and ventral halves. However, expressed as micrograms P^{31} per cell, a ventral-dorsal gradient was apparent. Data expressed as specific activity (counts per minute per microgram P^{31}) exhibit no significant differences.

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